Bacteriological evaluation of dog and cat diets that claim to contain probiotics

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Abstract — Nineteen commercial pet foods claiming to contain probiotics were evaluated. Selective bacterial culture was performed to identify organisms that were claimed to be present. Twelve diets claimed only to contain specific bacterial fermentation products, which does not necessarily indicate that live growth would be expected, but these products also included the term "probiotic" somewhere on the package, suggesting that live, beneficial organisms were present. No products contained all of the listed organisms, while 1 or more of the listed contents were isolated from 10 out of 19 products (53%). Eleven products contained additional, related organisms including *Pediococcus* spp, which was isolated from 4 products. No relevant growth was present in 5 (26%) products. Average bacterial growth ranged from 0 to 1.8×10^5CFU/g. Overall, the actual contents of the diets were not accurately represented by the label descriptions.

Résumé — Évaluation bactériologique de diètes pour chiens et chats alléguant contenir des probiotiques. Dix-neuf aliments commerciaux pour chiens et chats allèguant contenir des probiotiques ont été évalués. Une culture bactérienne sélective a été effectuée afin d'identifier les organismes prétendus présents. Douze diètes qui prétendaient contenir uniquement des produits spécifiques de la fermentation bactérienne, ce qui n'indiquait pas nécessairement qu'on pouvait s'attendre à une croissance bactérienne, faisaient aussi mentions du terme «probiotique» quelque part sur leur emballage, insinuant que des organismes vivants bénéfiques étaient présents. Aucun produit ne contenait tous les organismes énumérés alors qu'au moins un des organismes mentionnés a été isolé de 10 des 19 produits (53 %). Onze produits contenaient des organismes apparentés additionnels dont *Pediococcus* spp, isolé à partir de 4 produits. Aucune croissance pertinente n'a été décelée chez 5 (26 %) des produits. La croissance bactérienne moyenne allait de 0 à 18×10^5 UFC/g. Dans l'ensemble, l'étiquetage ne correspondaient pas exactement aux contenus réels des diètes.

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Introduction

Probiotics are microorganisms that, when ingested, exert beneficial effects beyond that of their nutritional value (1). Probiotic therapy is becoming increasingly popular in veterinary medicine; however, few results from objective research are available, particularly for dogs and cats. Probiotic therapy has been recommended for the treatment or prevention of a variety of conditions in different species. A number of probiotic products are available commercially for use in dogs and cats; they are available in tablet, capsule, paste, and liquid forms. Some commercial dog and cat foods also claim to contain probiotics. Incorporation of probiotics into diets may have the advantage of easy, daily administration of beneficial organisms.

Because probiotics are considered food supplements, not drugs, there are no regulations regarding their use as supplements or food additives. Various studies have reported that quality control among probiotic supplements intended for human or animal use is poor, with a significant percentage of products either not containing the organisms stated on the label, not containing the numbers of organisms stated on the label, or containing additional species (2-5) Studies evaluating the quality control of pet foods claiming to contain probiotics have not been reported. The goal of this study was to isolate, enumerate, and identify probiotic bacteria in pet foods claiming to contain probiotics.

Materials and methods

Commercially available pet (dog and cat) foods claiming to contain probiotics were purchased from local

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retailers. Products listing bacterial species in the ingredient list or claiming to contain 'probiotics' on the packaging were included. Lot numbers and expiry dates were recorded and all products were tested prior to their expiry date. Quantitative culture was performed on all products. Ten grams of food were added to 90 mL of phosphate buffered saline (PBS, pH 7.2) and homogenized in a blender. Serial 10-fold dilutions were performed in PBS and 100 μL of each dilution was inoculated onto deMan, Rogosa, Sharpe (MRS) agar and incubated anaerobically for the isolation of lactic acid bacteria, and onto blood agar and incubated aerobically and anaerobically for the isolation of enterococci and bacilli. All plates were incubated at 37°C for 48 h.

Enrichment culture was used to aid in the isolation of low levels of organisms that might be overlooked in quantitative culture. For enrichment, 500 mg of each food was added to 10 mL each of MRS broth for growth of lactic acid bacteria, tryptone soya broth for growth of aerobes, and brain heart infusion broth for growth of anaerobes. After 24 h of incubation, 100 μL of broth was inoculated onto MRS agar and incubated under anaerobic conditions for the isolation of lactic acid bacteria, onto blood agar under aerobic conditions for the isolation of aerobes, and onto blood agar under anaerobic conditions for the isolation of anaerobes. Plates were incubated at 37°C for 48 h. Organisms were identified via colonial morphology, Gram stain reaction, and biochemical characteristics. Lactobacilli were identified by using the API 50 CHL biochemical assay (Biomerieux, St. Laurent, Quebec) and bacilli were identified via standard biochemical tests (6). Enterococci were identified by using the API 20 Strep biochemical assay (Biomerieux). All testing was performed in duplicate. No attempt was made to identify or enumerate organisms unrelated to those listed on the labels.

Results

Nineteen diets were tested. Thirteen were for dogs and 6 were for cats. All claimed to contain specific organisms or probiotics. Two products listed 1 or more bacterial species as ingredients, while 12 listed fermentation products of probiotic species, and 5 listed both organisms and fermentation products. One product claimed to contain *Streptococcus faecium*, which was reclassified as *Enterococcus faecium* in 1984 (7). Five products misspelled 1 of the listed species.

Bacterial growth was present in all products; however, as the purpose of this study was to evaluate the contents of the diets compared with those claimed on the label, no attempt was made to identify organisms that were not included on the list of ingredients. No products contained all of the claimed organisms, while 1 or more of the listed contents were isolated from 10 out of 19 (53%) products (Table 1). Eleven products contained additional, related organisms, including *Pediococcus* spp, which were isolated from 4 products. Five (26%) products did not contain any relevant growth.

Discussion

Interpretation of these results is confounded somewhat by the questionable labelling of some products. Twelve diets listed only specific bacterial fermentation products (*L. acidophilus* fermentation product) as ingredients, while 5 diets claimed to contain both specific organisms and fermentation products. Fermentation products of lactic acid bacteria or bacilli are typically included as a source of enzymes. This does not necessarily indicate that live organisms are present and, based on the definition provided above, these would not be considered to be probiotics. However, the diets in this study that listed only fermentation products in the ingredients also stated directly that they contained probiotics. Therefore, the variable levels of live growth may better reflect misclassification or incorrect advertising of the diets, rather than indicate poor survival of probiotic organisms.

The diets that were tested contained between 0 and 1.8×10^5 CFU/g. It is unclear whether the level of supplementation present in these diets would be adequate, even if the organisms that were present possessed probiotic properties. Doses of 1×10^{10} to 1×10^{11} CFU/d are often used in human probiotic efficacy studies (8–10). Kailasapathy et al (11) have suggested a minimum therapeutic dose of 1×10^8 to 1×10^{10} CFU/d in humans, while others have claimed that at least 1×10^9 to 1×10^{10} viable organisms must reach the intestinal tract in humans to be effective (3,12,13). Dose requirements for probiotic organisms have not been evaluated adequately in dogs and cats, and they likely vary between probiotic strains. However, *Lactobacillus rhamnosus* strain GG was only variably isolated from the feces of dogs fed doses of 1×10^9 and 1×10^{10} CFU/d (Weese and Anderson, unpublished data). Approximately 5.5 kg/d of the diet containing the highest level of growth would be required to provide 1×10^9 CFU/d. It was interesting to note that bacterial names were misspelled on the labels of 5 products. While this does not necessarily indicate a poor quality product, such errors raise concerns.

Enterococcus faecium was the most commonly isolated organism and has been reported to have probiotic properties in vitro and in vivo (14,15). However, concern has been expressed over the use of enterococci, because they can be opportunistic pathogens and probiotic strains of enterococci are able to transfer the *vanA* gene; the gene responsible for vancomycin resistance (16,17). *Lactococcus lactis* was identified in 7 products, despite not being included on the label of any product, while *L. plantarum* was present in 4 diets. Certain strains of *L. plantarum* and *Lc. lactis* have been demonstrated to possess probiotic properties; however, it is unclear whether the strains isolated from these diets possessed any such properties (18,19). *Lactobacillus acidophilus* was purported to be present in 13 diets, yet it was not identified in any. This was surprising and an explanation is not readily apparent. *Lactobacillus acidophilus* has been isolated routinely from probiotic supplements and fecal samples by the author's laboratory, so it is unlikely that laboratory error accounted for this finding. Inaccurate identification by the biochemical assay could be considered; however, in 9 out of 14 (64%) of the diets claiming to contain *L. acidophilus*, no organisms isolated with a similar Gram stain appearance, let alone similar biochemical characteristics, were isolated. Despite being listed on the label of 3 products, *Bacillus*

Table 1. Comparison of actual bacterial probiotic contents versus label claims in commercial pet foods

Product	Label claim	Actual contents	Numbers (CFU/g)
1	*Lactobacillus acidophilus* fermentation product *Bifidobacterium thermophilum* fermentation product *Enterococcus faecium* fermentation product Yogurt	*Pediococcus pentosaceus* *Lactococcus lactis* *E. faecium*	1×10^3 1×10^3 2×10^4
2	*L. acidophilus* fermentation product *Streptococcus faecium* fermentation product	*P. pentosaceus*	1×10^2
3	*L. acidiphilus* (sic) fermentation product *S. faecium* fermentation product *Bacillus subtilis*	No growth[a]	N/A
4	*Bacillus subtillus* (sic) fermentation solubles *L. acidophilus*	*L. crispatus* *Lactobacillus* spp (non-speciated)	Enrichment only 7×10^2
5	*L. plantarum* *E. faecium* *L. casei* *L. acidophilus*	*L. plantarum*	2×10^2
6	*L. acidophilus* *E. faecium* *Bacillus subtilis* fermentation extract	No growth	N/A
7	*L. acinophilus* (sic) fermentation product *B. thermophilum* fermentation product *E. faecium* fermentation product Yogurt	*Lc. lactis*	Enrichment only
8	*L. acidophilus* fermentation product *E. faecium* fermentation product *L. plantarum* fermentation product *L. casei* fermentation product *Bifidobacterium bifidum* fermentation product *E. diacetylactis* fermentation product	*E. faecium* *L. plantarum* *Lc. lactis*	3×10^3 2×10^3 Enrichment only
9	*Bacillus licheniformis* fermentation extract *Bacillus subtilis* fermentation extract	*B. subtilis*	2×10^4
10	*L. acidophilus* *E. faecium* *Bacillus subtilis* fermentation extract	*Lc. lactis* *E. faecium*	Enrichment only 3×10^3
11	*Bacillus licheniformis* fermentation extract *Bacillus subtilis* fermentation extract	No growth	N/A
12	*L. acidophilus* *E. faecium* *B. subtilis* fermentation solubles	*L. plantarum* *E. faecium*	8×10^2 1.8×10^4
13	*L. acidophilus* *E. faecium* *B. subtilis* fermentation solubles	*Lc. lactis* *E. faecium*	1.5×10^5 1×10^4
14	*L. acidophilus* fermentation product *E. faecium* fermentation product *L. plantarum* fermentation product *L. casei* fermentation product *Bifidobacterium bifidum* fermentation product *E. diacetylactis* fermentation product	*E. faecium* *Lc. lactis*	6×10^3 1×10^3
15	*L. acidophilus*	*P. pentosaceus*	Enrichment only
16	*B. subtilis* fermentation extract	No growth	N/A
17	*Bacillus licheniformis* fermentation extract *Bacillus subtilis* fermentation extract	No growth	N/A
18	*Bacillus subtilis* fermentation product *Enterococcoccus* (sic) *faecium* fermentation product *L. acidophilus* fermentation product *L. casei* fermentation product *L. lactis* fermentation product	*E. faecium* *Pediococcus* spp *Lc. lactis*	1.8×10^5
19	*Bacillus subtilis* fermentation product *Entercococcus* (sic) *faecium* fermentation product *L. acidophilus* fermentation product *L. casei* fermentation product *L. lactis* fermentation product	*E. faecium* *L. plantarum*	1.3×10^4 8×10^4

[a]No growth of organisms claimed on the label, or related species
L — lactobacillus
Lc — lactococcus
Information regarding the products that were tested is available upon request

licheniformis was not isolated from any diet, and *B. subtilis* was identified in only 1 out of 12 (8.3%) diets. Once it was determined that the stated bacilli were not present, no further attempts were made to identify contents, so it is possible that other bacilli were present. Mislabeling of probiotics claiming to contain specific bacilli has been reported elsewhere (20,21). The isolation of *Pediococcus* spp. from 4 diets was surprising. Pediococci have not been reported to possess beneficial properties and are considered to be opportunistic pathogens (22). The failure to detect *Bifidobacterium* spp. from any product likely relates to the strict anaerobic nature of this genus (23) and is not surprising, as similar results have been reported in human and veterinary probiotic supplements (2,4,5).

Two products claimed to contain yogurt. This does not adequately indicate which bacterial species would be expected to be present. Most yogurt products contain *L. bulgaricus* and *Streptococcus thermophilus*, which are not regarded as being probiotics because they are poorly able to survive bile environments and are typically unable to colonize the intestinal tract (24,25). Yogurt containing probiotic strains is available, but there is no indication that probiotic-containing yogurt was added to these diets.

The culture techniques that were employed here would be expected to be adequate for isolation of all of the probiotic organisms listed on the labels. The use of both direct and enrichment culture techniques should have decreased the possibility that some organisms were overlooked. It is possible that some strains of similarly appearing lactobacilli were not individually identified. Representative colonies from plates containing differently appearing colonies were identified, but it cannot be ruled out that species with similar colonial and Gram stain appearances were not distinguished. This could have affected the results in diets with multiple organisms; however, it would not affect the overall bacterial counts.

The yeast component of the diets was not evaluated. Some species of yeast, notably *Saccharomyces boulardii*, have been shown to possess probiotic properties (26,27); however, most yeast additives act as nutritional supplements, not probiotics. There is no evidence that any of the yeast species listed on the labels of the tested diets possess probiotic properties.

Overall, commercial pet foods that claim to contain probiotics appear to contain very low numbers of viable organisms, and often do not contain the species listed on the label. Whether this relates to improper addition of organisms during processing, failure to survive processing, or poor viability during storage is unclear. Regardless of the contents of any diets containing lactic acid bacteria or bacilli, it is debatable as to whether they should be considered to contain probiotics without demonstration of species-specific efficacy. Based on extrapolation from other species, it seems likely that certain probiotics will be shown to be effective for the treatment or prevention of certain conditions in dogs and cats. Currently, there is very little information available regarding research on probiotic therapy in dogs. Results of this study indicate that these commercial diets are not good sources of probiotics. While production of a probiotic-containing diet should be possible, research must be performed to select bacterial species with beneficial in vitro and in vivo properties, and the ability to survive commercial processing and storage. CVJ

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BOOK REVIEW

COMPTE RENDU DE LIVRE

Tams T. ***Upper GI Endoscopy.*** Lifelearn, Guelph, ON, 2000, ISBN 1-8969-8536-X, CDN$99.00.

The CD ROM *Upper GI Endoscopy* teaches in an interactive format the principles of endoscopy of the upper gastrointestinal tract in dogs and cats. Five hours of continuing education (CE) credit can be earned by the completion and sending in of a test (additional US $15/CDN $19) at the end of the program. The CD ROM is written for the Microsoft Windows based PC. I tested the program on a 1Mhz Intel Celeron computer running Microsoft Windows XP Home edition, with a 40x CD ROM. From a technical point of view, the installation of the program is not perfect. For example, the program started to install itself, did not recognize a newer Quick-time version already present, and tried to install its own older version. Then it proceeded with the opening screen by registering you will receive, which was followed by an empty box instead of the list of benefits of registering. The program does not allow for full 1024 × 768 screen mode. The result is a smaller window in which the actual program runs; despite this limitation picture resolution is adequate. The quality of the speech varies and is better if no concurrent video is loading. One way to improve the sound quality is to replay the speech (there is a convenient button in all screens to do so) after each video section has loaded.

The CD is divided into several sections: Esophagoscopy, Gastroscopy, Duodenoscopy, and Biopsy/Brush Cytology. In addition, 3 Clinical Cases are presented in which it is demonstrated how endoscopy was helpful in reaching a diagnosis. The setup of the presentation is logical and each section can be studied on its own. A minor disadvantage of this approach is that if modules are taken one after the other, a lot of repeat information is shown, which can become tedious. The ability to maneuver around the learning module is good. Several direct paths leading to the desired segment of the module can be selected. The description of the actual endoscope movements is adequate. Within the program, regular reference is made to another CD ROM from Lifelearn, namely *Introduction to Flexible Endoscopy* where more instruction on handling and setup of the endoscope is provided. The description for the technique of performing an endoscopic examination is adequate but, at times, awkward. For example, the advice to complete a total gastroscopy prior to advancement into the duodenum is, in my opinion, ill advised; it will result in an increased chance of closure of the pylorus in response to the gastric distention and stimulation. Subsequent passage into the duodenum past the pyloric sphincter will become much more difficult compared with completing the gastroscopy once the duodenoscopy has been completed. In my opinion, to see an open pyloric sphincter during scoping (which stays open as happens in the video demonstration) is rare, a closed pyloric sphincter is much more common. The latter can be a real challenge to pass through with the endoscope, a subject not discussed in the program.

Some findings and interpretations of the clinical cases are open for discussion. For example, the finding of thickened bowel loops on abdominal palpation has, in clinical practice, been fraught with errors. In my opinion, only abdominal ultrasonography can be used as a noninvasive reliable, reproducible method of determining intestinal wall thickness. In another case, a dog with chronic vomiting, the use of H2 blocker therapy was rejected, despite the likely presence of mild esophagitis. On the other hand, the recommended use of sucralfate suspension in a patient with esophagitis is probably of little value. As always, it is advisable to check drug choices and dosages in standard reference text books prior to commencement of therapy. In summary: *Upper GI Endoscopy* provides an attractive interactive way of demonstrating endoscopy to practitioners. The CD ROM's main function will be to complement wet labs and standard endoscopy texts.

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